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# Impaired fear extinction retention and increased anxiety-like behaviours induced by limited daily access to a high-fat/high-sugar diet in male rats: Implications for diet-induced prefrontal cortex dysregulation

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ABSTRACT

Anxiety disorders and obesity are both common in youth and young adults. Despite increasing evidence that over-consumption of palatable high-fat/high-sugar "junk" foods leads to adverse neurocognitive outcomes, little is known about the effects of palatable diets on emotional memories and fear regulation. In the present experiments we examined the effects of daily 2 h consumption of a high-fat/high-sugar (HFHS) food across adolescence on fear inhibition and anxiety-like behaviour in young adult rats. Rats exposed to the HFHS diet exhibited impaired retention of fear extinction and increased anxiety-like behaviour in an emergence test compared to rats fed a standard diet. The HFHS-fed rats displayed diet-induced changes in prefrontal cortex (PFC) function which were detected by altered expression of GABAergic parvalbumin-expressing inhibitory interneurons and the stable transcription factor ΔFosB which accumulates in the PFC in response to chronic stimuli. Immunohistochemical analyses of the medial PFC revealed that animals fed the HFHS diet had fewer parvalbumin-expressing cells and increased levels of FosB/ΔFosB expression in the infralimbic cortex, a region implicated in the consolidation of fear extinction. There was a trend towards increased IBA-1 immunoreactivity, a marker of microglial activation, in the infralimbic cortex after HFHS diet exposure but expression of the extracellular glycoprotein reelin was unaffected. These findings demonstrate that a HFHS diet during adolescence is associated with reductions of prefrontal parvalbumin neurons and impaired fear inhibition in adulthood. Adverse effects of HFHS diets on the mechanisms of fear regulation may precipitate a vulnerability in obese individuals to the development of anxiety disorders.



## 1. Introduction

The modern diet of developed countries has become increasingly rich in refined sugars and saturated fats (Cordain et al., 2005; Kearney, 2010). The negative consequences of consumption of large amounts of this diet on physical health have been well studied (Basu, McKee, Galea, & Stuckler, 2013; Haslam & James, 2005). However, less is known about the effects of over-consumption of palatable high-fat/high-sugar (HFHS) foods on mental health. Recent studies indicate a role for diet quality in common psychological disorders such as anxiety. For example, epidemiological studies indicate that poor quality diets with higher intakes of processed foods are associated with increased prevalence of anxiety disorders (Jacka, Mykletun, Berk, Bjelland, & Tell, 2011). Despite this evidence, the mechanisms linking palatable high energy foods and anxiety are not well known.

Studies in adult rodents link intake of high energy foods to alterations in emotional regulation and anxiety-like behaviour. Although one study reported that very brief 10 min daily access to a sweet-fat diet for 2–3 weeks did not lead to increased anxiety-like behaviour in rats (Parylak, Cottone, Sabino, Rice, & Zorrilla, 2012), another study reported that longer exposures (i.e., 1 month of 12 h daily access) to palatable foods did result in rats showing increased anxiety-like behaviours on the elevated plus maze (EPM) when tested 24 h after the last exposure (i.e., when

*Abbreviations:* BLA, basolateral amygdala; CS, conditioned stimulus; DAB, 3′,3′-diaminobenzadine; EPM, elevated plus maze; HDL, high density lipoprotein; HFHS, High-fat/high-sugar; IBA-1, ionized calcium-binding adaptor molecule 1; IL, infralimbic; IR, immunoreactive; mPFC, medial prefrontal cortex; NHS, normal horse serum; P, postnatal day; PB, phosphate buffer; PBS, phosphate buffered saline; PFC, prefrontal cortex; PL, prelimbic; PV, parvalbumin; US, unconditioned stimulus; WAT, white adipose tissue.

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deprived, or withdrawn from the palatable diet) (Avena, Rada, & Hoebel, 2008; Colantuoni et al., 2002). These findings suggest that chronic (i.e., greater than 1 month) intermittent access to high energy foods may increase anxiety-like behaviour.

The effects of chronic intermittent access to high energy foods on emotion regulation are likely driven by diet-induced neurobiological changes. Neurobiological changes following regular consumption of palatable foods can lead to enduring adaptations in the neural circuits responsible for emotion regulation (Boitard et al., 2015) as well as reward circuits (Johnson & Kenny, 2010). Specifically, alterations to prefrontal cortical and amygdala function after the consumption of diets high in fat or sugar is proposed to underlie pronounced neurocognitive changes to emotional learning (Boitard et al., 2015; Reichelt, Maniam, Westbrook, & Morris, 2015) and deficits in behavioural control in cognitive tasks dependent upon the prefrontal cortex (PFC), including decision making (Reichelt, Killcross, Hambly, Morris, & Westbrook, 2015), appetitive blocking (Sharpe, Clemens, Morris, & Westbrook, 2016), prepulse inhibition (Labouesse, Stadlbauer, Langhans, & Meyer, 2013; Wakabayashi, Numakawa, Ooshima, Hattori, & Kunugi, 2015), and working memory (McNeilly, Williamson, Sutherland, Balfour, & Stewart, 2011). For example, it was reported that three months of consumption of a high-fat diet from weaning, but not adulthood, resulted in enhanced cued fear learning and altered synaptic plasticity in the amygdala of rats (Boitard et al., 2015). Such findings suggest that exposure to a high-fat diet leads to augmentation of learned fear responses.

Very little is known about the effects of consumption of palatable HFHS foods on fear inhibition. Learned fear can be inhibited through the process of extinction, an important component of exposure-based therapies for anxiety disorders. During extinction, repeated exposure to the conditioned stimulus (CS) in the absence of the aversive unconditioned stimulus (US) diminishes fear responses elicited by the CS. Extinction is not simply erasure of the original fear memory, but is thought to involve the formation of a new inhibitory (CS-noUS) association. The consolidation of extinction is critically dependent upon interactions of the medial PFC (mPFC) and amygdala, particularly those projections from the infralimbic (IL) subregion of the mPFC to the basolateral amygdala (BLA) (Bukalo et al., 2015; Do-Monte, Manzano-Nieves, Quinones-Laracuente, Ramos-Medina, & Quirk, 2015; Milad & Quirk, 2002). However, extinction is not always permanent. In both rodents and humans extinguished fear responses often recover under a number of conditions as a consequence of the competing fear learning and extinction memories. In particular, fear responses are very prone to relapse after extinction when the PFC is maturing during the adolescent developmental stage (Baker, Bisby, & Richardson, 2016; Baker, Den, Graham, & Richardson, 2014; Pattwell et al., 2012). Given that a failure to appropriately inhibit, or extinguish, fear is a key feature of anxiety disorders (Otto, Smits, & Reese, 2004), it is critical to understand whether environmental factors such as diet might increase the likelihood of impaired fear inhibition. It is currently unknown whether chronic intermittent access to highly palatable foods impairs the acquisition or retention of fear extinction.

In this study we examined the effects of 2 h daily HFHS diet consumption on anxiety-like behaviour and fear inhibition in young adult rats. We used two complementary behavioural tests measuring the avoidance of potentially threatening situations and the extinction of learned fear. Anxiety-like behaviour was measured 24 h after access to the HFHS diet to determine whether limited 2 h daily HFHS diet access evokes anxiety-like behaviour after a longer duration exposure period (i.e., after 6 weeks) than in previous studies (Colantuoni et al., 2002; Parylak et al., 2012). PFC function was assessed by examining immunohistochemical markers of several proteins relating to inhibitory neurons, chronic PFC activation, and microglial activity. We focused on parvalbumin (PV)-containing neurons, a major class of GABAergic interneurons, which are essential elements of the circuits involved in various forms of learning. In the mPFC PV neurons are known to regulate fear expression through their perisomatic inhibition of pyramidal neurons (Courtin et al., 2014). A further reason was that PV neurons are integral to high-order behavioural control and functionally mature in the PFC during adolescence (Morishita, Kundakovic, Bicks, Mitchell, & Akbarian, 2015; O'Donnell, 2011). We also counted the number of PV neurons in the BLA given that IL inputs to the BLA are necessary for formation of extinction memories (Bukalo et al., 2015). Further, recent studies have identified that PV interneurons in the BLA control fear learning (Wolff et al., 2014). In the mPFC we compared expression of the stable transcription factor ΔFosB across diet conditions as a measure of chronic PFC activity (Chen, Kelz, Hope, Nakabeppu, & Nestler, 1997; McClung et al., 2004). ΔFosB has previously been used as a marker of neuronal activation in response to repeated exposure to rewarding stimuli (Ohnishi et al., 2015; Renthal et al., 2008). In addition, we examined expression of the plasticity-regulating glycoprotein reelin, secreted by GABAergic neurons, which is posited to play a major role in the development of neuropsychiatric disorders such as schizophrenia (Campo, Sinagra, Verrier, Manzoni, & Chavis, 2009) as well as cognition (Brigman, Padukiewicz, Sutherland, & Rothblat, 2006). As a final dependent measure we considered potential effects of a HFHS diet on immune activity within the brain. We measured ionized calcium-binding adaptor molecule 1 (IBA-1) as a marker of microglia, which can reflect changes in microglial activity (Kreutzberg, 1996). Increased IBA-1 expression has been observed following high energy diet consumption and is associated with impaired metabolic function (Thaler & Schwartz, 2010; Valdearcos et al., 2014; Yi, Tschop, Woods, & Hofmann, 2012). Thus, we hypothesised that limited daily access to a HFHS diet starting in adolescence would induce anxiety-like behaviour, impair the extinction of learned fear, and induce neurobiological changes to the PFC in adult male rats.

## 2. Methods

### 2.1. Subjects

Male (N = 24) albino Sprague Dawley rats (supplied by Animal Resources Centre, Western Australia) were delivered to animal house facility at the School of Psychology, UNSW Australia at 3 weeks of age soon after weaning. Rats were randomly allocated to boxes and housed in groups of four in plastic cages with wire tops (dimensions: 60 cm [length] × 40 cm [width] × 26 cm [height]) in a climate controlled (21 °C ± 2 °C; humidity 55 ± 5%) colony room illuminated on a 12 h light-dark cycle (lights on at 07:00 h). Standard laboratory rat chow (Gordon's Specialty Stockfeeds, NSW, Australia, 65% carbohydrates, 12% fat and 23% protein, with an energy density of 11 kJ/g) and water was available *ad libitum* throughout the experiment. All experiments were carried out between 09:00 and 17:00 h. All procedures were approved by the Animal Care and Ethics Committee at UNSW Australia and conducted in accordance with the guidelines of the *Australian Code of Practice for the Care and Use of Animals for Scientific Purposes* (National Health and Medical Research Council, 2004).

A timeline of the general experimental procedures is described in Fig. 1. The diet administration began on postnatal day (P) 28, coinciding with widely held definitions of the timing of adolescence in rats from P28 to ~P50-55 (Schneider, 2013; Spear, 2000; Spear & Swartzwelder, 2014).